

We claim:

1. A regulated polymerase III expression system, comprising
  - (a) a first nucleic acid segment comprising a regulated promoter
  - operably linked to a first element encoding a transcription factor; and
  - (b) a second nucleic acid segment comprising a recombinant polymerase III promoter regulated by the transcription factor,wherein the transcription factor increases transcription from the recombinant polymerase III promoter.
2. The expression system of claim 1, wherein binding of the transcription factor to (i) the polymerase III promoter or to (ii) at least one binding site operably linked to the polymerase III promoter increases transcription from the recombinant polymerase III promoter.
3. The expression system of claim 1, wherein the first and second nucleic acid segments reside in the same nucleic acid.
4. A nucleic acid comprising the first and second nucleic acid segments of claim 1.
5. The nucleic acid of the preceding claim comprising the nucleic acid sequence as set forth in SEQ ID NO: 1.
6. A nucleic acid comprising the nucleic acid sequence as set forth in SEQ ID NO: 1.
7. A nucleic acid comprising the nucleic acid sequence as set forth in SEQ ID NO: 2.
8. A cell comprising the regulated polymerase III expression system of claim 1.
9. A non human organism comprising the cell of claim 8.

10. A non human organism comprising the regulated polymerase III expression system of claim 1.
- 5 11. The expression system of the claim 1, wherein the regulated promoter is an inducible promoter.
12. The expression system of claim 11, wherein transcription from the inducible promoter is increased in the presence of an ecdysone, an ecdysone-analog or  
10 an ecdysone mimic.
13. The expression system of claim 11, wherein transcription from the inducible promoter is increased by muristerone A.
- 15 14. The expression system of claim 11, wherein transcription from the inducible promoter is increased by tetracycline or an agonist thereof.
15. The expression system of claim 1, wherein transcription from the regulated promoter is developmentally regulated.  
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16. The expression system of the claim 1, wherein transcription from the regulated promoter is tissue specific.
17. The expression system of the claim 1, wherein transcription from the  
25 regulated promoter is temporally regulated.
18. The expression system of the claim 1, wherein transcription from the regulated promoter is cell-cycle regulated.
- 30 19. The expression system of claim 1, wherein the regulated promoter comprises or is operably linked to at least one ecdysone response element.

20. The expression system of claim 1, wherein the transcription factor comprises a DNA-binding domain and a transactivating domain.
21. The expression system of the preceding claim, wherein the DNA-binding  
5 domain is a GAL4 DNA-binding domain.
22. The expression system of claim 1, wherein the DNA-binding domain does not comprise a tet DNA-binding domain.
- 10 23. The expression system of claim 20, wherein the transactivating domain is an Oct-1 or an Oct-2 domain.
24. The expression system of claim 20, wherein the transactivating domain is an Oct-2<sup>Q</sup>(Q→A) domain.
- 15 25. The expression system of claim 20, wherein the transcription factor binds to at least one binding site operably linked to the polymerase III promoter.
26. The expression system of claim 1, wherein the transcription factor does not  
20 bind an inducer.
27. The expression system of the preceding claim, wherein the inducer is tetracycline or doxycycline.
- 25 28. The expression system of claim 1, wherein expression of the transcription factor is dependent on the presence of an inducer.
29. The expression system of claim 1, wherein transcription from the recombinant polymerase III promoter is dependent on the presence of an  
30 inducer.
30. The expression system of the preceding claim, wherein the transcription

factor regulates transcription from the recombinant RNA polymerase III promoter by binding to (i) at least one binding site operably linked to said promoter; or (ii) to said promoter.

- 5     31.     The expression system of the preceding claim, wherein binding of the transcription factor to the recombinant RNA polymerase promoter by or to a binding site operably linked to said promoter increases transcription from said promoter.
- 10    32.     The method of claim 29, wherein binding affinity of the transcription factor for (i) the polymerase III promoter or for (ii) the binding site operably linked to said promoter is substantially the same in the presence or absence of the inducer.
- 15    33.     The expression system of claim 1, wherein the polymerase III promoter is a mammalian promoter.
34.     The expression system of claim 1, wherein the polymerase III promoter element comprises a U6 promoter or an H1 promoter.
- 20    35.     The expression system of claim 1, wherein the polymerase III promoter comprises a TATA box.
36.     The method of the preceding claim, wherein the TATA box comprises the sequence TATAAA.
- 25    37.     The expression system of claim 1, wherein the second nucleic acid segment comprises at least one binding site for the transcription factor operably linked to the recombinant polymerase III promoter.
- 30    38.     The expression system of the preceding claim comprising four binding sites for the transcription factor, operably linked to the recombinant polymerase

III promoter.

39. The expression system of claim 1 comprising a number of binding sites for the transcription factor which result in the highest signal-to-noise ratio for transcription from the recombinant polymerase III promoter.
40. The expression system of claim 1 wherein transcription from the recombinant polymerase III promoter is dependent on the presence of an inducer.
41. The expression system of the preceding claim, wherein the transcription factor regulates transcription from the recombinant RNA polymerase III promoter by binding to (i) a binding site operably linked to said promoter; or (ii) to said promoter.
42. The expression system of the preceding claim, wherein binding affinity of the transcription factor for (i) the polymerase III promoter or for (ii) the binding site operably linked to said promoter is substantially the same in the presence or absence of the inducer.
43. The expression system of claim 1, wherein the regulated promoter is further operably linked to a second element.
44. The expression system of the preceding claim, wherein the second element encodes a reporter protein, a selectable marker or an enzyme.
45. The expression system of the preceding claim, wherein the reporter protein comprises a fluorescent protein.
46. The expression system of the preceding claim, wherein the fluorescent protein comprises a GFP protein.
47. The expression system of the preceding claim, wherein the selectable marker

comprises a cell surface receptor or a drug-resistance marker.

- 5           48.     The expression system of claim 43, wherein the second element encodes a second transcription factor, a transcriptional activator or a transcriptional repressor.
49.     The expression system of the claim 43, wherein the second element encodes a protein that regulates transcription from the recombinant polymerase III promoter or from the regulated promoter.
- 10           50.     The expression system of claim 1, further comprising a sequence of a transgene operably linked to the recombinant polymerase III promoter.
51.     The expression system of claim 50, wherein the transgene encodes a non-coding RNA.
- 15           52.     The expression system of claim 51, wherein the non-coding RNA comprises an siRNA.
53.     The expression system of claim 51, wherein the transgene comprises a hairpin RNA.
- 20           54.     The expression system of claim 51, wherein the transgene comprises a ribozyme.
55.     The expression system of the preceding claim, wherein the ribozyme comprises a Cech-type ribozyme or a hammerhead ribozyme.
- 25           56.     The expression system of claim 51, wherein the non-coding RNA inhibits the expression of an essential gene.
- 30           57.     The expression system of claim 1, further comprising a cloning site

downstream of the polymerase III promoter.

58. The expression system of the preceding claim, wherein the cloning site comprises a restriction enzyme recognition site or a ccdB sequence.
59. The expression system of claim 57, wherein the position of the cloning site relative to the polymerase III promoter allows transcription of a DNA sequence inserted into the cloning site from the recombinant polymerase III promoter.
60. The expression system of claim 1, comprising at least one nucleic acid segment encoding a regulatory protein which promotes transcription from the regulated promoter.
61. The expression system of the preceding claim, comprising a nucleic acid segment encoding two regulatory proteins which promote transcription from the regulated promoter.
62. The expression system of claim 1, further comprising at least two nucleic segments wherein each of the nucleic acids segments encodes a regulatory protein which promotes transcription from the regulated promoter.
63. The expression system of claim 60, wherein the regulatory protein binds to an inducer.
64. The expression system of the preceding claim, wherein binding of the regulatory protein to the inducer promotes transcription from the regulated promoter.
65. The expression system of the claim 63, wherein binding of the regulatory protein to the inducer promotes binding of the regulatory protein to a response element.

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66. The expression system of claim 60, wherein the regulatory protein binds to the regulated promoter or to a response element operably linked to the regulated promoter.
67. The expression system of the preceding claim, wherein binding of the regulatory protein to the regulated promoter or to a response element operably linked to the regulated promoter promotes transcription from the regulated promoter.
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68. The expression system of claim 60, wherein the regulatory protein does not bind to the polymerase III promoter.
69. The expression system of claim 60, wherein the regulatory protein comprises a DNA binding domain.
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70. The expression system of the preceding claim, wherein the DNA-binding domain of the regulatory protein comprises a tet repressor DNA binding domain, an RxR DNA binding domain or a nuclear hormone receptor DNA binding domain.
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71. The expression system of claim 60, wherein the regulatory protein promotes transcription from the regulated promoter upon binding to an inducer.
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72. The expression system of the preceding claim, wherein the inducer is tetracycline, ecdysone hormone, or an agonist thereof.
73. The expression system of claim 60, wherein the protein is a nuclear receptor or a transcription factor.
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74. The expression system of the preceding claim, wherein the protein comprises a VgEcR or an RXR protein.



75. A method of reducing gene expression of a gene in a cell, the method comprising
- (a) providing a cell comprising
- 5 (i) a regulated promoter operably linked to a first element encoding a transcription factor; and
- (ii) a recombinant polymerase III promoter regulated by the transcription factor and operably linked to a coding sequence for an RNA molecule, wherein expression of the RNA
- 10 molecule reduces expression of the gene; and
- (b) contacting the cell with an inducer, wherein the inducer promotes transcription of the RNA molecule from the recombinant polymerase III promoter,
- thereby reducing expression of the gene in the cell.
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76. A method of determining the effects of reducing gene expression of a gene in a cell, the method comprising
- (a) providing a cell comprising
- 20 (i) a regulated promoter operably linked to a first element encoding a transcription factor; and
- (ii) a recombinant polymerase III promoter regulated by the transcription factor and operably linked to a coding sequence for an RNA molecule, wherein expression of the RNA
- 25 molecule reduces expression of the gene;
- (b) subjecting the cell to a condition which promotes transcription of the RNA molecule from the recombinant polymerase III promoter; and
- (c) determining the phenotype of the cell;
- thereby determining the effects of reducing expression of the gene.
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77. A method of determining the effects of reducing gene expression of a gene in an organism, the method comprising
- (a) providing an organism wherein at least a cell in the organism comprises

- (i) a regulated promoter operably linked to a first element encoding a transcription factor; and
- (ii) a recombinant polymerase III promoter regulated by the transcription factor and operably linked to a coding sequence for an RNA molecule, wherein expression of the RNA molecule reduces expression of the gene;
- (b) subjecting the organism to conditions which promote transcription of the RNA molecule from the recombinant polymerase III promoter in at least one cell; and
- (c) determining the phenotype of at least one cell in the organism; thereby determining the effects of silencing expression of a gene in an organism.
78. The method of any one of claims 75-77, wherein the regulated promoter is an inducible promoter.
79. The method of the preceding claim, wherein transcription from the inducible promoter is increased in the presence of an ecdysone, an ecdysone analog or an ecdysone mimic.
80. The method of any one of claims 75-77, wherein the transcription factor does not comprise a tet DNA binding domain.
81. The method of any one of claims 75-77, wherein the transcription factor does not bind an inducer.
82. The method of the preceding claim, wherein the inducer is tetracycline or doxycycline.
83. The method of any one of claims 75-77, wherein expression of the transcription factor is dependent on the presence of an inducer.

84. The method of anyone of claims 75-77, wherein transcription from the recombinant polymerase III promoter is dependent on the presence of an inducer.
- 5 85. The method of the preceding claim, wherein the transcription factor regulates transcription from the recombinant RNA polymerase III promoter by binding to (i) a binding site operably linked to said promoter; or (ii) to said promoter.
- 10 86. The method of claim 77, wherein binding affinity of the transcription factor for (i) the polymerase III promoter or for (ii) the binding site operably linked to said promoter is substantially the same in the presence or absence of the inducer.
- 15 87. The method of claim 77, wherein binding of the transcription factor to the recombinant RNA polymerase promoter or to a binding site operably linked to said promoter increases transcription from said promoter.
- 20 88. The method according to any one of claims 75-77, wherein the RNA molecule is an shRNA molecule or an siRNA molecule.
89. The method of claim 75 or 76, wherein the cell is in an organism.
90. The method of claim 75, wherein the cell is a stem cell.
- 25 91. The method of the preceding claim, wherein the organism is an animal, a plant or a fungus.
92. The method of the preceding, wherein the animal is a mammal.
- 30 93. The method of the preceding claim, wherein the mammal is a mouse.
94. The method of any one of claims 75-77, wherein the RNA molecule

comprises an shRNA or an siRNA.

95. A method of reducing expression of a gene in a cell, the method comprising
- 5 (a) providing a cell comprising the regulated polymerase III expression system of anyone of the preceding claims, wherein the recombinant polymerase III promoter is operably linked to a coding sequence for an RNA molecule, wherein expression of the RNA molecule reduces expression of the gene; and
- 10 (b) contacting the cell with an inducer, wherein the inducer promotes transcription of the RNA molecule from the recombinant polymerase III promoter,
- thereby reducing expression of the gene in the cell.
96. A cell comprising the regulated polymerase expression system according to
- 15 any one of the preceding claims.